# High-Dose Amphotericin B with Flucytosine for the Treatment of Cryptococcal Meningitis in HIV-Infected Patients: A Randomized Trial

**Tihana Bicanic,[1,4] Robin Wood,[1] Graeme Meintjes,[2,3] Kevin Rebe,[2,3] Annemarie Brouwer,[4,6] Angela Loyse,[1,4] Linda-Gail Bekker,[1] Shabbar Jaffar,[5] and Thomas Harrison[1,4]**

[1]Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine, and [2]Department of Medicine, University of Cape Town, and [3]HIV Service, GF Jooste Hospital, Cape Town, South Africa; [4]Centre for Infection, Department of Cellular and Molecular Medicine, St. George's University of London, and [5]Department of Epidemiology and Population, London School of Hygiene and Tropical Medicine, London, United Kingdom; and [6]Department of Internal Medicine and Infectious Diseases, University Medical Centre Nijmegen, Nijmegen, The Netherlands

**(See the editorial commentary by Powderly on pages 131–2)**

***Background.*** The standard therapy for human immunodeficiency virus (HIV)–associated cryptococcal meningitis of amphotericin B (AmB; 0.7 mg/kg per day) plus flucytosine frequently takes >2 weeks to sterilize the cerebral spinal fluid, and acute mortality remains high. A dosage range for AmB of 0.7–1 mg/kg per day is noted in current guidelines, but there are no data comparing 0.7 mg/kg per day with 1 mg/kg per day.

***Methods.*** Sixty-four HIV-seropositive, antiretroviral therapy–naive patients in Cape Town, South Africa, who experienced their first episode of cryptococcal meningitis during the period May 2005–June 2006 were randomized to receive either (1) AmB, 0.7 mg/kg per day, plus flucytosine, 25 mg/kg 4 times per day (group 1; 30 patients); or (2) AmB, 1 mg/kg per day, plus flucytosine, 25 mg/kg 4 times per day (group 2; 34 patients). Regimens were given for 2 weeks, followed by treatment with oral fluconazole. The primary outcome measure was early fungicidal activity, as determined by results of serial, quantitative cerebral spinal fluid cryptococcal cultures. Secondary outcome measures were safety and mortality. The median duration of follow-up was 1 year.

***Results.*** Early fungicidal activity was significantly greater for group 2 than for group 1 (mean ± SD, $-0.56 \pm 0.24$ vs. $-0.45 \pm 0.16$ log cfu/mL of cerebral spinal fluid per day; $P = .02$). The incidence of renal impairment did not significantly differ between the 2 groups. Anemia was associated with female sex and, less strongly, with membership in group 2. Renal impairment and anemia reversed after the regimen was switched to fluconazole. Two- and 10-week mortality rates were 6% and 24%, respectively, with no difference between groups.

***Conclusions.*** AmB, 1 mg/kg per day, plus flucytosine is more rapidly fungicidal than is standard-dose AmB plus flucytosine. Because of its size, this study provides limited data on any difference in toxicity between the regimens, but toxicities were manageable and reversible.

***Clinical trials registration number.*** ISRCTN68133435 (http://www.controlled-trials.com).

Cryptococcosis is a common fungal opportunistic infection in patients with AIDS. In the parts of Africa where there is a high HIV seroprevalence, *Cryptococcus neoformans* is the leading cause of adult meningitis, and infection accounts for a high proportion of deaths in HIV-infected cohorts [1–4].

Amphotericin B (AmB), the cornerstone of antifungal therapy for cryptococcal meningitis (CM), has concentration-dependent activity [5]. Since the 1980s, clinical trials have used progressively increasing doses of AmB. More recent trials have studied a dosage of 0.7 mg/kg per day, yielding better results than did previous studies of lower dosages [6–9]. Despite these developments, acute mortality due to CM remains high, with the 10-week mortality rate for recipients of AmB-based therapies ranging from 10% in a US study, which excluded the most-severe cases [9], to 33% in a South

Received 23 November 2007; accepted 27 February 2008; electronically published 27 May 2008.

Reprints or correspondence: Dr. Tihana Bicanic, Div. of Infectious Diseases, Dept. of Cellular and Molecular Medicine, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, United Kingdom (tihana@never.com).

**Clinical Infectious Diseases 2008; 47:123–30**

1058-4838/2008/4701-0021$15.00
DOI: 10.1086/588792

Presented in part: 17th European Congress of Clinical Microbiology and Infectious Diseases, Munich, Germany, April 2007 (abstract O169).

African series that did not exclude patients with decreased consciousness level [10]. Furthermore, the current standard treatment regimen (AmB, 0.7 mg/kg per day, plus flucytosine, 100 mg/kg per day [11]) still takes >2 weeks to sterilize the CSF in a significant proportion of patients [9]. Sterilization at 2 weeks has been shown to be associated with positive outcome at 10 weeks [12].

In a randomized trial performed in 2002 in Thailand, we showed that the rate of clearance of infection, or "early fungicidal activity" (EFA), is a powerful means of discriminating the activity of alternative antifungal regimens for HIV-associated CM. AmB (0.7 mg/kg per day in all treatment arms) plus flucytosine (100 mg/kg per day) was significantly more rapidly fungicidal than was AmB given alone, AmB plus fluconazole (400 mg per day), or triple-drug therapy with AmB, flucytosine, and fluconazole [7]. The 2-week regimen of AmB plus flucytosine was well tolerated in this study, as it was in the larger Mycoses Study Group trial [9], suggesting it may be possible, in this patient group, to further increase the dosage of AmB for a 2-week duration of therapy.

AmB (at 1 mg/kg per day) is commonly used in practice for many fungal infections and has been administered with flucytosine for treatment of CM for the initial 2 weeks of the regimen, with good effect and tolerability but without any comparative data [13]. On the premise that more-rapidly active, safe antifungal regimens should be associated with reduced acute mortality, the objective of this study was to determine the fungicidal activity and safety of relatively high-dosage conventional AmB (1 mg/kg per day) plus flucytosine, compared with standard-dosage AmB (0.7 mg/kg per day) plus flucytosine, to examine whether further increasing the dosage of AmB would lead to a measurable increase in the rate of CSF sterilization without unacceptable toxicity.

## METHODS

***Participants.*** The study was conducted from May 2005 to June 2006 at GF Jooste Hospital in Cape Town, South Africa, a secondary-level hospital serving an urban population of 1.3 million people with an estimated antenatal prevalence of HIV infection of 33% [14]. The study was approved by the research ethics committees of the University of Cape Town and St. George's Hospital (London, UK), as well as the Medicines Control Council of South Africa (Pretoria), and was conducted in accordance with the principles of the Helsinki Declaration of 1975, as revised in 1983. The study was registered as ISRCTN68133435 (step 1; http://www.controlled-trials.com).

HIV-infected patients (age, $\geqslant$18 years) who were hospitalized with a first episode of CM were eligible for enrollment. CM was diagnosed by India ink staining of CSF specimens. The diagnosis of CM was confirmed by a CSF culture positive for *C. neoformans.* Patients were excluded if they had an alanine aminotransferase level >5 times the upper limit of normal (>200 IU/L), an absolute neutrophil count $<500 \times 10^6$ cells/L, or a platelet count $<50{,}000 \times 10^6$ platelets/L; if they were pregnant or lactating; if they had previously experienced a serious reaction to AmB or flucytosine; or if they were already receiving antiretroviral therapy. Written informed consent was obtained from each patient or from the next-of-kin for patients with altered mental status (Glasgow Coma Scale, <15).

***Interventions.*** Patients were randomized in blocks of 8, using a computer-generated random sequence to provide numbers in sealed envelopes prepared by an independent person, to 1 of 2 treatment arms: group 1, AmB deoxycholate (Fungizone; Bristol-Myers Squibb), 0.7 mg/kg per day, plus oral flucytosine (currently not registered in South Africa; Valeant Pharmaceuticals, California), 25 mg/kg 4 times per day, for 2 weeks; or group 2, AmB, 1 mg/kg per day, plus flucytosine, 25 mg/kg 4 times per day, for 2 weeks. Treatment was not blinded. Randomization was stratified on the basis of altered mental status at the time of study admission, to try to ensure an equal number of severely ill patients in each treatment arm. After 2 weeks, patients in both arms received fluconazole (Diflucan; Pfizer), 400 mg per day for 8 weeks and 200 mg per day thereafter.

Unless contraindicated, patients received 1 L of 0.9% (normal) saline daily, to minimize AmB nephrotoxicity. Potassium and magnesium supplements were provided as required. If the serum creatinine increased to >2.5 mg/dL (220 $\mu$mol/L), despite administration of adequate hydration and saline loading, AmB and flucytosine were discontinued, and the regimen was switched early to fluconazole (400 mg per day). The flucytosine dose was adjusted for creatinine clearance in accordance with standard protocols [15].

Follow-up lumbar punctures were performed on days 3, 7, and 14 of treatment. Patients with CSF opening pressure >35 cm $H_2O$ and/or headache or other symptoms attributable to elevated pressure underwent additional lumbar punctures. After hospital discharge, participants were counselled and commenced antiretroviral therapy from week 4 after the start of antifungal therapy, and they were followed-up for 1 year after enrollment.

***Evaluation and outcomes.*** All participants underwent baseline blood testing for hematologic data, determination of electrolyte levels, assessment of renal and liver function, and determination of the CD4 cell count and HIV load; in addition, they underwent subsequent alternate-day renal function assessment and twice-weekly hematologic and liver function tests to monitor for adverse effects. The final serum creatinine or hemoglobin level was the last value obtained during the 2-week treatment period. Peak and trough values were the highest and lowest values, respectively, at any point during the treatment course. Percentage change of a value was calculated using the

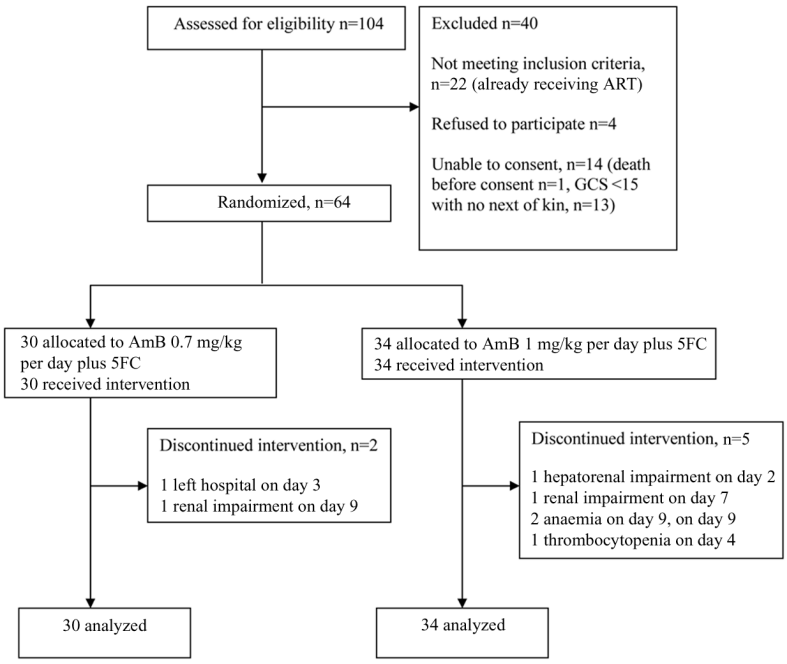


**Figure 1.** Profile of a trial of high-dose amphotericin B (AmB) with flucytosine (5FC) for the treatment of cryptococcal meningitis in HIV-infected patients. ART, antiretroviral therapy; GCS, Glasgow Coma Scale.

following formula: ([final value − baseline value]/baseline value) × 100%.

CSF samples were analyzed to determine the cell count and differential, protein and glucose levels, and cryptococcal antigen titer and underwent India ink staining and quantitative fungal culturing, as described elsewhere [7]. Cryptococcal clearance rates were calculated using a summary statistic for each patient, defined as the decrease in log cfu/mL of CSF per day, using the slope of the linear regression of log cfu against time for each patient [7].

The primary outcome measure was the mean rate of decrease in the number of *Cryptococcus* colony-forming units in the CSF or EFA for each treatment arm. Secondary outcome measures were rates of renal impairment and anemia, mortality at 2 and 10 weeks, and long-term survival during antiretroviral therapy.

***Sample size and statistics.*** In the initial study that used this end point, EFA was 74% faster for recipients of AmB, 0.7 mg/kg per day, plus flucytosine (0.54 log cfu/mL of CSF per day), compared with recipients of AmB, 0.7 mg/kg per day, alone (0.31 log cfu/mL of CSF per day) [7]. SDs for EFA in the 4 treatment groups ranged from 0.13 to 0.19. Using an SD of 0.19, 30 patients per arm yielded 90% power to detect a ⩾30% improvement in EFA in the experimental-treatment arm, compared with the standard treatment of AmB, 0.7 mg/kg per day, plus flucytosine, at an $\alpha$ level of 0.05 in a 2-sided test.

We compared baseline characteristics and outcomes in groups, using the $\chi^2$ test or Fisher's exact test for categorical variables, and we used the Mann-Whitney *U* test for continuous variables. Percentage changes in creatinine and hemoglobin levels in treatment groups were compared using Student's *t* test, and percentage changes in hemoglobin level were compared using linear regression, with adjustments made for sex as indicated. Linear regression was used to compare mean rates of decrease in the log cfu or EFA by treatment group, with adjustment as indicated for baseline organism load, giving summary differences with 95% CIs and significance levels [7]. Analyses were performed using Stata software, version 8 (Stata Corp.), and GraphPad Prism, version 4.03 (GraphPad Software).

## RESULTS

During the period May 2005–June 2006, 64 patients with India ink stains and CSF cultures positive for *C. neoformans* were enrolled in the study (figure 1): 30 were randomized to receive AmB, 0.7 mg/kg per day, plus flucytosine (group 1), and 34 were randomized to receive AmB, 1 mg/kg per day, plus flucytosine (group 2). The median duration of follow-up of survivors was 12 months (interquartile range, 11–13 months). One patient was lost to follow-up at 10 weeks, and 3 were lost to follow-up at 1 year.

Baseline clinical and laboratory characteristics and clinical outcomes are shown in table 1. At time of presentation with CM, 54 patients (84%) were known to be HIV seropositive. The median CD4 cell count was $38 \times 10^6$ cells/L, and the me-

**Table 1. Baseline clinical and laboratory characteristics and clinical outcomes for recipients of amphotericin B plus flucytosine.**

| Characteristic | All patients ($n$ = 64) | Group 1 ($n$ = 30) | Group 2 ($n$ = 34) | $P$ |
|---|---|---|---|---|
| No. (%) of men | 24 (38) | 15 (50) | 9 (26) | .07 |
| Age, years | 33 (28–38) | 34 (30–39) | 31 (28–37) | .17 |
| Weight, mean kg ± SD | 54 ± 10 | 53 ± 9 | 54 ± 11 | .75 |
| No. (%) of patients with known HIV infection at presentation | 54 (84) | 27 (90) | 27 (79) | .31 |
| No. (%) of patients with abnormal mental status | 8 (13) | 3 (10) | 5 (15) | .71 |
| CD4 cell count, $\times 10^6$ cells/L | 38 (12–69) | 35 (15–72) | 39 (10–57) | .36 |
| HIV load, copies/mL | 150,000 (48,000–540,000) | 160,000 (51,000–540,000) | 140,000 (47,000–520,000) | .76 |
| CSF data | | | | |
| Opening pressure, cm $H_20$ | 21 (14–30) | 22 (18–31) | 20 (10–29) | .22 |
| WBC count, cells/$mm^3$ | 19 (1–67) | 24 (5–80) | 17 (1–55) | .59 |
| Baseline fungal burden, CFU/mL of CSF | 174,750 (21,875–681,250) | 167,750 (30,375–433,750) | 174,750 (19,063–856,250) | .60 |
| Death, no. (%) of patients | | | | |
| By week 2 | 4 (6) | 1 (3) | 3 (9) | .62 |
| By weeks 10[a] | 15 (24) | 6 (21) | 9 (26) | .77 |

**NOTE.** Data are median (interquartile range), unless otherwise indicated. Group 1 received amphotericin B, 0.7 mg/kg per day, plus flucytosine; group 2 received amphotericin B, 1 mg/kg per day, plus flucytosine. Comparisons were performed using Student's $t$ test, the Mann-Whitney $U$ test, and Fisher's exact test.

[a] One patient was lost to follow-up at 10 weeks.

dian HIV load was 150,000 copies/mL. Eight patients (13%) had altered mental status (Glasgow Coma Scale, <15) at presentation. There were no significant differences between the 2 arms with regard to adverse prognostic factors for CM (abnormal mental status, baseline fungal burden, CSF WBC count, and CSF opening pressure) or severity of HIV infection (CD4 cell count and HIV load). There was a trend toward a greater number of women in group 2.

***EFA.*** The rate of clearance of infection during the first 2 weeks of therapy was more rapid for group 2 than for group 1. The mean EFA (±SD) was −0.56 ± 0.24 log cfu/mL of CSF per day for group 2 and −0.45 ± 0.16 log cfu/mL of CSF per day for group 1 (figure 2).

Rate of clearance was associated with AmB dose (difference, 0.11 log cfu per day; 95% CI, 0–0.22 log cfu/mL of CSF per day; $P = .05$) and, as was noted in a previous independent data set [7], with baseline CSF cfu count. In a linear regression model including treatment group and baseline count, both factors remained independently associated with rate of clearance: EFA was significantly greater for group 2 than for group 1 (difference, 0.12 log cfu/mL of CSF per day; 95% CI, 0.02–0.23 log cfu/mL of CSF per day; $P = .02$). Additional factors, including sex and CD4 cell count, were not associated with rate of clearance, and adjustment for additional factors did not make a significant difference to this final model.

***Mortality.*** The mortality rate was 6% (4 of 64 patients) at 2 weeks and 24% (15 of 63 patients) at 10 weeks, with no difference between groups. Sixty-eight percent and 60% of patients were alive at 6 months and 1 year, respectively, of follow-up. There was no difference in survival rates between the 2 groups at any time point.

***Safety.*** Both treatment regimens were well tolerated. There were no statistically significant differences between groups 1 and 2 in measurements of renal impairment (table 2). No patients required dialysis. As previously reported [16–18], anemia was common: a decrease in the hemoglobin level >2 g/dL developed in 50% and 71% of patients in groups 1 and 2, respectively ($P = .2$). In univariate analysis, both treatment group and sex, but not baseline hemoglobin level, were associated with the percentage decrease in the hemoglobin level. The percentage decrease in the hemoglobin level was greater for group 2 (difference, 9%; 95% CI, 2%–15%; $P = .01$) (table 2) and greater for women (difference, 10%; 95% CI, 4%–17%; $P = .002$). In multivariate analysis that included both of these variables, the percentage decrease in the hemoglobin level remained associated with sex (difference, 9%; 95% CI, 2%–15%; $P = .01$), but the association with treatment group was of borderline statistical significance (difference, 6%; 95% CI, 0.1%–13%; $P = .05$).

There was evidence of reversibility of renal impairment and anemia after the regimen was switched to fluconazole. For the 34 patients (14 from group 1 and 20 from group 2) for whom follow-up creatinine and hemoglobin data were available at 4 weeks (2 weeks after cessation of treatment with AmB plus flucytosine), the median creatinine levels at baseline, week 2, and week 4 were 65, 104, and 81 μmol/L, respectively (figure 3), and the median hemoglobin levels at baseline, week 2, and week 4 were 10.4, 7.7, and 8.5 g/dL, respectively. At week 4, the median creatinine level was 82 μmol/L in group 1 and 79 μmol/L in group 2, and the median hemoglobin level was 8.5 g/dL for both groups 1 and 2.

Six (9%) of 64 patients discontinued use of study drug(s)

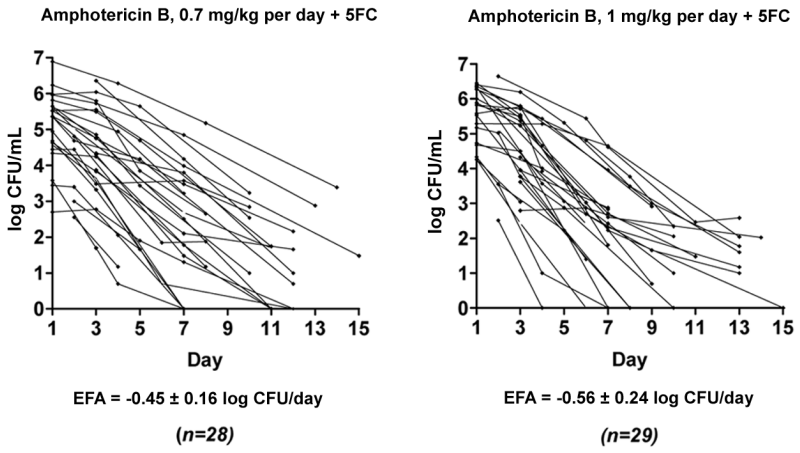


**Figure 2.** Decrease in number of CSF *Cryptococcus neoformans* colony-forming units over time by treatment group. The decrease in log cfu/mL CSF per day was calculated for each patient using the slope of the linear regression of log cfu against time. For each treatment group, early fungicidal activity (EFA) is shown as the mean ± SD rate in the decrease in log cfu counts. The EFA was greater for amphotericin B, 1 mg/kg per day, plus flucytosine (5FC) than for amphotericin B, 0.7 mg/kg per day, plus 5FC ($P = .02$, by linear regression).

before week 2 because of adverse reactions (table 2). Two patients (1 in each group) experienced increases in the creatinine level, to 229 $\mu$mol/L for the patient in group 1 (on day 7) and 231 $\mu$mol/L for the patient in group 2 (on day 9). One patient (in group 2) developed severe hepatorenal impairment (the serum creatinine level increased from 64 to 392 $\mu$mol/L, and the alanine aminotransferase level increased from 33 to 890 IU/L) after receiving a single dose of AmB plus flucytosine. Treatment with trimethoprim-sulfamethoxazole had been started simultaneously. The results of hepatitis serologic tests were negative. The patient received fluconazole once liver function had normalized and is alive (while receiving antiretroviral therapy) at 1 year. Two patients (both in group 2) stopped use of study drugs on day 9 because of anemia (hemoglobin levels, 4.8 and 5.5 g/dL). The former patient received a 2-U blood transfusion; the latter had iron deficiency and received supplementation. Additional measurements of the hemoglobin level at week 4 were 10 and 7.7 g/dL, respectively. Both patients are alive and receiving antiretroviral therapy at 1 year. Flucytosine alone was discontinued because of thrombocytopenia (the baseline platelet count was $60{,}000 \times 10^6$ platelets/L but decreased to $26{,}000 \times 10^6$ platelets/L) on day 4 in 1 patient (group 2); the platelet count increased after initiation of antiretroviral therapy, suggesting that the patient had HIV-related immune thrombocytopenia.

## DISCUSSION

Increasing the dosage of AmB from 0.7 to 1 mg/kg per day, given in combination with flucytosine, resulted in significantly more rapid clearance of cryptococcal infection from the CSF. This finding was demonstrated despite the relatively small number of patients studied. Interpretation of this finding may be helped by work, currently in progress, to determine any association between rate of clearance of infection and clinical outcome, by combining cohorts of patients studied using serial quantitative CSF cultures.

AmB-induced nephrotoxicity is thought to be mediated by a combination of decreased renal blood flow and increased tubular membrane permeability [19, 20] and is generally reversible, although complete resolution may take weeks or even months [21]. Published studies of conventional AmB deoxycholate (0.7 mg/kg per day for ⩾2 weeks) to treat HIV-associated CM report rates of renal impairment of up to 50%, although definitions of renal impairment vary [9, 16, 22, 23]. In this study, the rates of renal impairment were comparable to those reported by previous studies; also, differences in measurements of renal impairment between the 2 groups did not reach statistical significance, although this may have been related to the relatively small size of the study: 13% and 32% of patients in groups 1 and 2, respectively, had increases in the creatinine level of >2-fold from the baseline level, compared with 33% of patients in the study by Hamill et al. [22]. Patients in both groups experienced some increase in the creatinine level, but this was not difficult to manage, and the increases had improved by the first outpatient visit 2 weeks after discharge. Few patients (3 of 64) discontinued AmB treatment prematurely because of renal impairment; for those who did, the discontinuation usually occurred during the second week of treatment.

Anemia associated with AmB therapy is a common—although perhaps not so widely appreciated—phenomenon, developing in up to 75% of AmB recipients [16, 18]. It is typically normochromic and normocytic and may result, at least in part, from suppression of erythropoietin production [24] through

**Table 2. Treatment and laboratory characteristics for patients during the initial 2 weeks of therapy.**

| Parameter | Group 1 ($n$ = 30) | Group 2 ($n$ = 34) | $P$ |
|---|---|---|---|
| No. of patients who discontinued use of the study drug early | 1 | 5 | .20 |
| Baseline creatinine level, median $\mu$mol/L (IQR) | 62 (57–77) | 67 (54–79) | .74 |
| Final creatinine level >2 × the baseline level | 4 (13) | 11 (32) | .09 |
| Peak creatinine level >3 × the baseline level | 3 (10) | 5 (15) | .71 |
| Increase in creatinine level, mean % ± SD | 26 ± 30 | 34 ± 28 | .32 |
| Baseline hemoglobin level, median g/dL (IQR) | 11.1 (9.6–12.2) | 10.2 (8.7–11.6) | .12 |
| Decrease in the hemoglobin level >2 g/dL | 15 (50) | 22 (71) | .2 |
| Decrease in hemoglobin level, mean % ± SD | −16 ± 12 | −25 ± 12 | .01 |

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. Group 1 received amphotericin B, 0.7 mg/kg per day, plus flucytosine; group 2 received amphotericin B, 1 mg/kg per day, plus flucytosine. Comparisons were performed using Student's $t$ test, the Mann-Whitney $U$ test, and Fisher's exact test. IQR, interquartile range.

inactivation of a key transcription factor regulating EPO gene expression [25]. In one prior study of HIV-associated cryptococcosis, AmB treatment (0.7 mg/kg per day) was associated with a mean decrease in the hemoglobin level of 3 g/dL at 2 weeks, with 59% of patients receiving transfusions [17]. The percentage decreases in the hemoglobin level in our study were similar to the 23% decrease in the hemoglobin level seen in a Thai study of AmB given at a dosage of 0.7 mg/kg per day [26]. The decrease in the hemoglobin level at the end of therapy was greater in women than in men, suggesting that women may be more vulnerable to anemia induced by AmB. The decrease in the hemoglobin level at the end of therapy was also higher in group 2 than in group 1, although this difference had resolved 2 weeks after the switch to fluconazole and was largely explicable by the association of the decrease in hemoglobin with the patients's sex, because there was a higher proportion of women in group 2. Nevertheless, the possibility of severe anemia in the short term is a concerning adverse effect in Africa, where blood products and facilities for safe transfusion may be limited. Administration of a single dose of long-acting exogenous erythropoietin may be an interesting option to try to prevent severe anemia [27] and may be necessary only for persons with a low baseline hemoglobin level. Studies would be needed to demonstrate the efficacy of this treatment, because an acute inflammatory response may reduce the effectiveness of exogenous erythropoietin [28].

In this trial of AmB-based combination therapy, the 10-week survival rate was 76%, the highest reported to date from Africa and higher than the rates in previous studies of fluconazole from Africa [29–31]; it is also slightly higher than the 67% 10-week survival rate in a prior cohort of patients from the same center who were treated initially with AmB alone for a median duration of 1 week [10]. Of note, the earlier studies of fluconazole were performed before there was access to antiretroviral therapy, but there is no evidence that initiation of antiretroviral therapy, as practiced to date, has decreased the acute, 10-week rate of mortality due to cryptococcal disease [10, 32]. The results of the current trial were achieved despite inclusion of severely ill patients: 13% of patients had a reduced conscious level, the most important predictor of poor prognosis [6]. In addition, the 6- and 12-month survival rates for patients with access to antiretroviral therapy, which were based on almost complete follow-up data, demonstrated that, once the patient recovers from acute cryptococcal infection and starts receiving antiretroviral therapy, the prognosis is good for patients with CM in Africa, as in the developed world. This good long-term prognosis underscores the need to explore antifungal drug dosages and combinations and adjunctive treatment strategies to

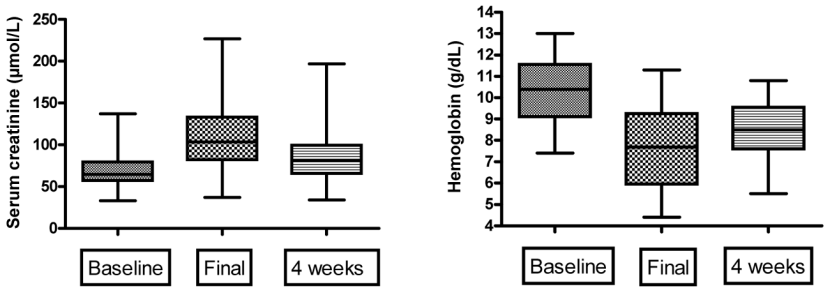


**Figure 3.** Box-and-whiskers plot of creatinine and hemoglobin levels at baseline, week 2 (end of induction treatment), and at 2 weeks after discharge for all patients for whom 4-week laboratory values were available ($n$ = 34).

try to reduce the proportion of patients with CM who die before 10 weeks. Currently, this proportion is still between one-quarter and one-half of patients.

On the basis of its more-rapid clearance of infection and manageable toxicity, we favor the use of AmB at a dosage of 1 mg/kg per day plus flucytosine for 2 weeks, provided that laboratory data are monitored carefully and that transfusion is possible if it is occasionally needed. If significant toxicity occurs, it usually happens during the second week of treatment, and patients can be switched early to fluconazole at a point when the cryptococcal CSF cfu count has been reduced by $\geqslant$4 log cfu. In a follow-up study examining the best second drug to pair with AmB, AmB is being administered at a dosage of 1 mg/kg per day. AmB at 1 mg/kg per day is also being studied in another large trial of the treatment of CM in Vietnam (ISRCTN95123928; http://www.controlled-trials.com). If the results of these ongoing studies demonstrate that flucytosine remains the best second drug, initiatives to improve access to flucytosine in Africa and Asia will be needed [33].

## Acknowledgments

Sister Nomqondiso Sidibana was the research nurse and Xhosa interpreter for the study. We thank all the patients participating in this study, as well as the doctors, nurses, and HIV counselors at the GF Jooste Hospital Infectious Diseases Clinic, medical wards and Carnation ward, and the staff at the Desmond Tutu HIV Centre at the University of Cape Town for their support and assistance in the conduct of this study.

***Financial support.*** British Infection Society (fellowship grant to T.B.) and Medical Research Council (UK) (project grant 76201).

***Potential conflicts of interest.*** All authors: no conflicts.

## References

1. Corbett EL, Churchyard GJ, Charalambos S, et al. Morbidity and mortality in South African gold miners: impact of untreated disease due to human immunodeficiency virus. Clin Infect Dis **2002**; 34:1251–8.
2. French N, Gray K, Watera C, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS **2002**; 16:1031–8.
3. Hakim JG, Gangaidzo IT, Heyderman RS, et al. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. AIDS **2000**; 14:1401–7.
4. Okongo M, Morgan D, Mayanja B, Ross A, Whitworth J. Causes of death in a rural, population-based human immunodeficiency virus type 1 (HIV-1) natural history cohort in Uganda. Int J Epidemiol **1998**; 27: 698–702.
5. Andes D. Pharmacokinetics and pharmacodynamics of antifungals. Infect Dis Clin North Am **2006**; 20:679–97.
6. Saag MS, Powderly WG, Cloud GA, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. The NIAID Mycoses Study Group and the AIDS Clinical Trials Group. N Engl J Med **1992**; 326:83–9.
7. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. Lancet **2004**; 363:1764–7.
8. Larsen RA, Leal MA, Chan LS. Fluconazole compared with amphotericin B plus flucytosine for cryptococcal meningitis in AIDS: a randomized trial. Ann Intern Med **1990**; 113:183–7.
9. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. N Engl J Med **1997**; 337:15–21.
10. Bicanic T, Meintjes G, Wood R, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. Clin Infect Dis **2007**; 45:76–80.
11. Saag MS, Graybill RJ, Larsen RA, et al.; Infectious Diseases Society of America. Practice guidelines for the management of cryptococcal disease. Clin Infect Dis **2000**; 30:710–8.
12. Robinson PA, Bauer M, Leal MA, et al. Early mycological treatment failure in AIDS-associated cryptococcal meningitis. Clin Infect Dis **1999**; 28:82–92.
13. de Lalla F, Pellizzer G, Vaglia A, et al. Amphotericin B as primary therapy for cryptococcosis in patients with AIDS: reliability of relatively high doses administered over a relatively short period. Clin Infect Dis **1995**; 20:263–6.
14. Western Cape Department of Health. The 2005 Antenatal HIV provincial and area surveys, Western Cape. Cape Town, South Africa: Provincial Government of the Western Cape, **2005**.
15. Daneshmend TK, Warnock DW. Clinical pharmacokinetics of systemic antifungal drugs. Clin Pharmacokinet **1983**; 8:17–42.
16. Joly V, Aubry P, Ndayiragide A, et al. Randomized comparison of amphotericin B deoxycholate dissolved in dextrose or Intralipid for the treatment of AIDS-associated cryptococcal meningitis. Clin Infect Dis **1996**; 23:556–62.
17. Sharkey PK, Graybill JR, Johnson ES, et al. Amphotericin B lipid complex compared with amphotericin B in the treatment of cryptococcal meningitis in patients with AIDS. Clin Infect Dis **1996**; 22:315–21.
18. Brandriss MW, Wolff SM, Moores R, Stohlman F Jr. Anemia induced by amphotericin B. JAMA **1964**; 189:663–6.
19. Deray G. Amphotericin B nephrotoxicity. J Antimicrob Chemother **2002**; 49(Suppl 1):37–41.
20. Gallis HA, Drew RH, Pickard WW. Amphotericin B: 30 years of clinical experience. Rev Infect Dis **1990**; 12:308–29.
21. Butler WT, Bennett JE, Alling DW, Wertlake PT, Utz JP, Hill GJ. Nephrotoxicity of amphotericin B: early and late effects in 81 patients. Ann Intern Med **1964**; 61:175–87.
22. Hamill RJ, Sobel J, El-Sadr W. Randomized double-blind trial of AmBisome (liposomal amphotericin B) and amphotericin B in acute cryptococcal meningitis in AIDS patients [abstract 1161]. In: Program and abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco). Washington, DC: American Society for Microbiology, **1999**.
23. Leenders AC, Reiss P, Portegies P, et al. Liposomal amphotericin B (AmBisome) compared with amphotericin B both followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. AIDS **1997**; 11:1463–71.
24. Lin AC, Goldwasser E, Bernard EM, Chapman SW. Amphotericin B blunts erythropoietin response to anemia. J Infect Dis **1990**; 161: 348–51.
25. Yeo EJ, Ryu JH, Cho YS, et al. Amphotericin B blunts erythropoietin response to hypoxia by reinforcing FIH-mediated repression of HIF-1. Blood **2006**; 107:916–23.
26. Brouwer AE, van Kan HJ, Johnson E, et al. Oral versus intravenous flucytosine in patients with human immunodeficiency virus-associated cryptococcal meningitis. Antimicrob Agents Chemother **2007**; 51: 1038–42.
27. Lancaster DJ, Palte S, Ray D. Recombinant human erythropoietin in the treatment of anemia in AIDS patients receiving concomitant amphotericin B and zidovudine. J Acquir Immune Defic Syndr **1993**; 6: 533–4.
28. Kwack C, Balakrishnan VS. Managing erythropoietin hyporesponsiveness. Semin Dial **2006**; 19:146–51.
29. Mwaba P, Mwansa J, Chintu C, et al. Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. Postgrad Med J **2001**; 77:769–73.

30. Mayanja-Kizza H, Oishi K, Mitarai S, et al. Combination therapy with fluconazole and flucytosine for cryptococcal meningitis in Ugandan patients with AIDS. Clin Infect Dis **1998**; 26:1362–6.
31. Schaars CF, Meintjes GA, Morroni C, Post FA, Maartens G. Outcome of AIDS-associated cryptococcal meningitis initially treated with 200 mg/day or 400 mg/day of fluconazole. BMC Infect Dis **2006**; 6:118.
32. Lortholary O, Poizat G, Zeller V, et al. Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. AIDS **2006**; 20:2183–91.
33. Bicanic T, Wood R, Bekker LG, Darder M, Meintjes G, Harrison TS. Antiretroviral roll-out, antifungal roll-back: access to treatment for cryptococcal meningitis. Lancet Infect Dis **2005**; 5:530–1.